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Interaction between proteasomal and lysosomal systems can be modulated to reduce $A\beta_{42}$ effects in hippocampal slice cultures

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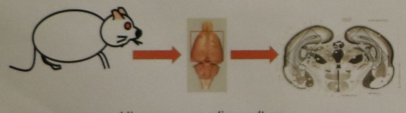
Abstract

Intracellular protein clearance decreases with age, thus altering the vital balance between protein synthesis vs. degradation and initiating a slow cascade of protein accumulation events in the brain. Typically, accumulating toxic protein species lead to the activation of proteasomal and lysosomal pathways for clearing such species. However, many studies have indicated that the two pathways exhibit stress during protein accumulation disorders such as Alzheimer's disease. In this study, low concentration $A\beta_{42}$ was applied to hippocampal slice cultures resulting in reduced proteasome activity (p=0.0058) in correspondence with increased tau phosphorylation, as well as a significant loss of synaptophysin. When the slice cultures were treated with the proteasome inhibitor lactacystin, a nearly complete and rapid reduction in proteasome activity was found. Interestingly, during the proteasomal compromise a putative compensatory response by the lysosomal enzyme cathepsin B (CatB) was detected, as evident by a 50-75% increase in CatB activity. This suggests potential cross-talk between proteasomes and lysosomes as previously suggested (Pandey et al. 2007). To further assess the apparent inverse relationship, we used a lysosomal enhancing agent (Z-Phe-Ala-diazomethylketone; PADK) that promotes protein clearance (Butler et al. 2011; Bahr et al. 2012) in order to test for proteasomal attenuation. However, PADK did not attenuate proteasomes. Surprisingly, PADK appeared to cause a small increase in proteasome activity (control = 98.7±1.7, PADK = 115.3±12.4, N.S.). In slices with Aβ42-mediated proteasomal compromise (34.7±5.2% of control), PADK indeed enhanced this proteasome activity to 73.0±10.0% of control (p=0.027), thus to levels comparable to those found in control slices. Furthermore, PADK also reduced Aβ42-mediated tau phosphorylation by >50%, an event previously suspected to involve lysosomal compromise, and more recently implicated as a consequence of the negative influence on proteasomes and protein clearance efficiency. Such efficiency may involve cross-talk between proteasomes and lysosomes. Together, the results suggest a distinct interaction between proteasomal and lysosomal systems, and they point to potential dual modulation against protein accumulation pathology linked to Alzheimer's disease and other dementias.

1.The slice culture method provides mature brain tissue exhibiting native neuronal organization



Hippocampus slice culture

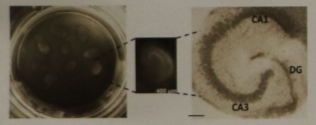


Figure 1. Transverse slices of hippocampus (400 µm) were placed on the Biopore PTFE membrane of culture insert in a culture plate of 6 wells with media. Illustrative image of synaptic circuits of the hippocampus network show the different subregions: DG, CA3 and CA1 (view-field width: 2.8 mm).

2. Low-concentration $A\beta_{42}$ causes proteasome stress, synaptic decline in correspondence with increased p-Tau levels in hippocampal slice cultures

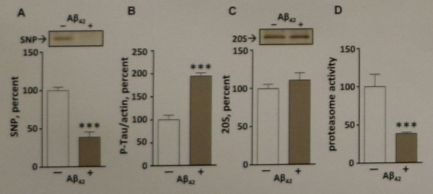


Figure 2. Aliquots of human $A\beta_{1-42}$ peptide were allowed to self-aggregate in PBS for 6 days before being applied daily to slice cultures at 0.5-1.5 µM alongside vehicle-treated slices. The tissue was harvested after 6 days, sonicated, and equal protein aliquots assessed by immunoblot for synaptophysin (SNP), phospho-tau (Ser^{199}/Ser^{202}), 20S proteasome (20S) and actin as a load control. Integrated optical densities were tabulated for SNP (A), phospho-tau (B) and 20s (C), and normalized to their respective controls and percent ± SEM values are shown. Unpaired t-tests. ***p< 0.001. In addition, hippocampal slices treated with $A\beta_{1-42}$ for 4-6 days were evaluated for proteasome chymotrypsin-like activity (D) (Vmax/s ± SEM; n=6 groups). Unpaired t-tests. ***p< 0.001.

3. Lactacystin reduced proteasome activity by 80% while promoted an increase on Cathepsin B (CatB) activity in hippocampal slice cultures

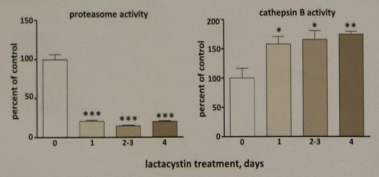


Figure 3. The slice cultures were treated daily with vehicle or with 5 µM lactacystin for 4 days, then fluorogenic peptide assays assessed the harvested slice samples for proteasome chymotrypsin-like and cathepsin B activities. The two measures were normalized to their respective vehicle control groups and percent ± SEM values are shown. ANOVA one-way: * p<0.05, **p<0.01 and *** p<0.0001 compared to control.

4. Low-level $A\beta_{42}$ also reduces proteasome activity followed by a compensatory increase in CatB activity

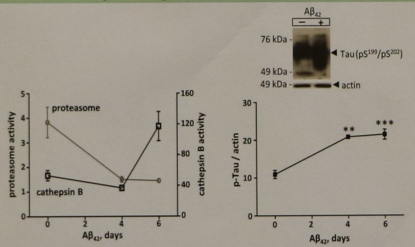


Figure 4. Hippocampal slice cultures were treated daily with vehicle for 6 days (0-day control groups) or with 1.5 µM pre-aggregated $A\beta_{42}$ for 4-6 days, staggering the treatments in order for same-day harvesting. Slice groups were sonicated to measure enzymatic activities in equal protein aliquots (30 µg) including proteasome chymotrypsin-like activity (gray plot of mean Vmax/s ± SEM; ANOVA: p<0.01) and cathepsin B activity (black plot of mean fluorometric units ± SEM; ANOVA: p<0.01). In addition, hippocampal slice cultures were treated daily with vehicle for 6 days or with 1.5 µM pre-aggregated $A\beta_{42}$ for 6 days. The slice samples were harvested, sonicated and equal protein aliquots assessed by immunoblot with antibody to phospho-tau (Ser^{199}/Ser^{202}) and actin. Integrated optical densities were tabulated for 55-68-kDa phospho-tau and their within-sample ratios with actin measures were plotted (normalized means ± SEM; n=6). ANOVA one-way: **p< 0.01, ***p< 0.001 compared to control.

Table 1. Compensatory Response Triggers for CatB Enhancement

Aβ42-induced proteasomal stress in hippocampal slice cultures	(Farizatto et al. submitted)
Lactacystin-induced proteasomal stress in hippocampal slices	(Farizatto et al. submitted)
Proteasome stress in young but not old rats – brain-injected lactacystin	(Gavilán et al. 2015)
Aβ42 treatment of N2a cells, but not by Aβ40	(Mueller-Steiner et al. 2006)
Reversible proteasome inhibitor in cells with APPwt or APP-Val717Gly	(Cecarini et al. 2014)
hAPP expression in mice at 1-3, 7-8, but not 16-20 months of age	(Mueller-Steiner et al. 2006)
Chloroquine-induced protein accumulation stress in hippocampal slices	(Bendiske & Bahr, 2003)
hAPP-N2a cells treated with phosphodiesterase III inhibitor which reduces Aβ42	(Park et al. 2016)
Huntingtin Htt-552 expression in PC12 cells	(Wu et al. 2012)
Traumatic brain injury model: increased CatB and CatB activity	(Luo et al. 2010)

5. $A\beta_{42}$ reduces proteasome activity and increases p-Tau levels, however both were attenuated by CatB enhancement agent

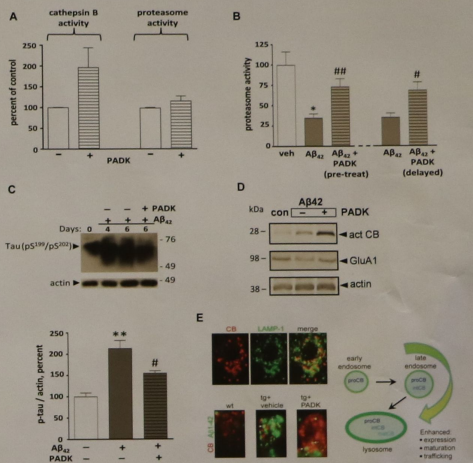


Figure 5. Hippocampal slice cultures were treated daily with vehicle or with 3 µM PADK for 2 days, then fluorogenic peptide assays assessed the harvested slice samples for cathepsin B and proteasome chymotrypsin-like activities. The two measures were normalized to their respective vehicle control groups (mean ± SEM) (A). In addition, a new set of slice cultures were treated daily with vehicle (veh) or 1.5 µM pre-aggregated $A\beta_{42}$ for 6 days, or pre-treated with 3 µM PADK for 1 day before starting the daily $A\beta_{42}$ treatments in combination with PADK (left bars). Alternatively, the 6-day $A\beta_{42}$ treatment was compared to similarly treated slices in which the PADK inclusion was delayed, being added to only the last 3 $A\beta_{42}$ treatments (right bars). Proteasome chymotrypsin-like activity used a fluorogenic peptide assay in harvested samples and Vmax/s measures were normalized to vehicle-treated samples (mean ± SEM). Unpaired t tests: compared to vehicle control *p=0.0196; compared to $A\beta_{42}$ alone: #p<0.05, ##p=0.027 (B). Also, phospho-Tau (Ser^{199}/Ser^{202}), actin, cathepsin B (CB) and GluA1 were assessed by immunoblot (C-D). hippocampal slice cultures were treated daily with vehicle (0-day control) or with 1.5 µM pre-aggregated $A\beta_{42}$ for 4-6 days in the absence or presence of 3 µM PADK. Integrated optical densities were tabulated for 55-68-kDa phospho-tau and normalized to their respective vehicle control groups and percent ± SEM values are shown. Unpaired t tests: compared to vehicle control **p<0.01; compared to $A\beta_{42}$ alone #p<0.05 (C). Hippocampal tissue from PADK-treated mice (intraperitoneally, 20 mg/kg per day×9 days) exhibiting increased mature cathepsin B levels was stained with anti-CB (red) and anti- lysosomal-associated membrane protein 1 (LAMP1) (green). The increase in organellar CB (red) in PADK-treated APPswe/PS1ΔE9 transgenic mice (tg) was associated with a decrease in intracellular $A\beta_{1-42}$ in CA1 pyramidal neurons (E). View-field widths, =12 µm. wt, Vehicle-treated wild-type mice.

Conclusion

In sum, the findings support that interactions occur between the ubiquitin-proteasome system and the lysosomal degradation pathway of autophagy during episodes of protein accumulation stress. Together, the two pathways make up a complimentary network for cell homeostasis, and modulated balancing between the two pathways through compensatory responses is a likely cellular attribute for maintaining consistent clearing of old and mis-folded proteins.

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Acknowledgments

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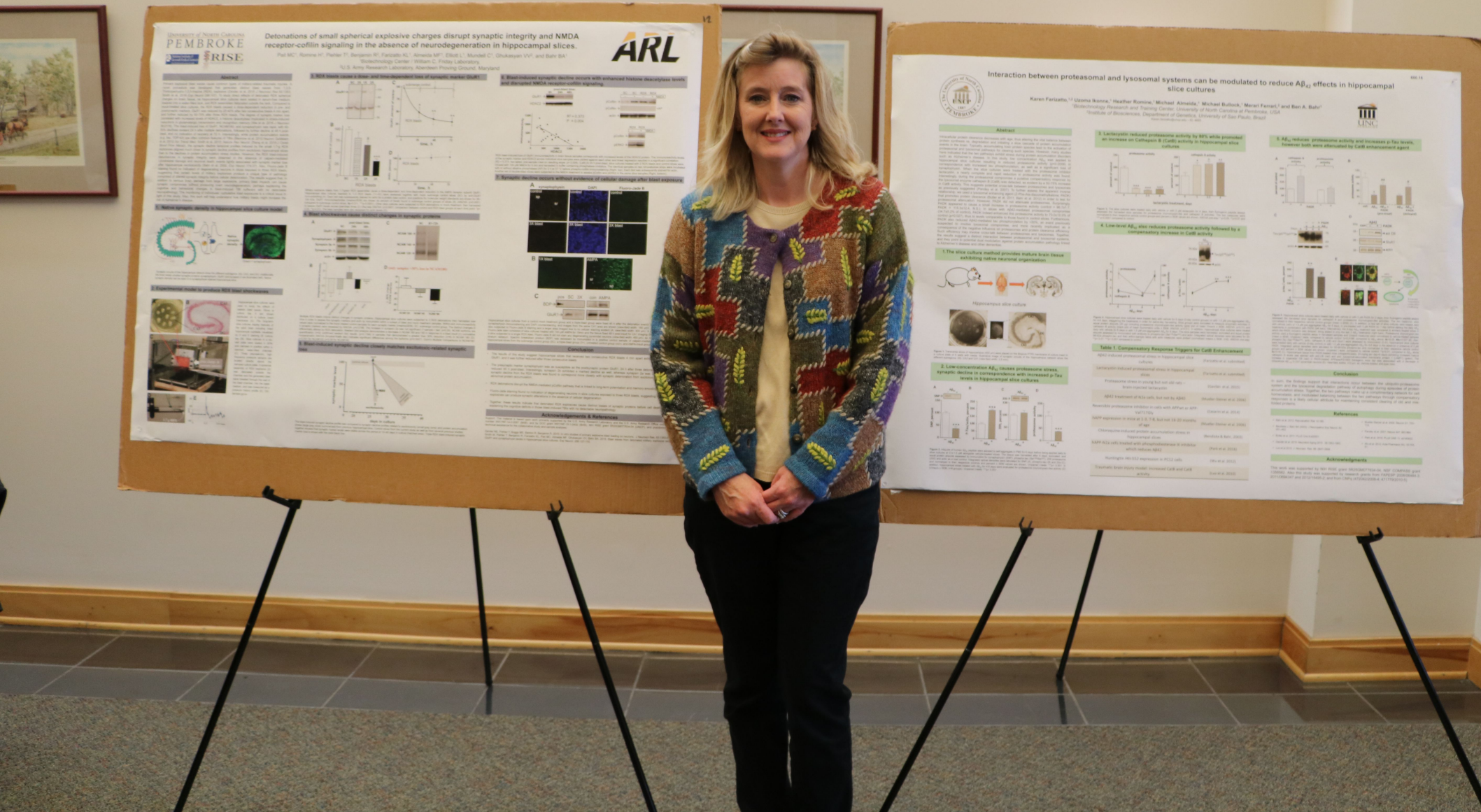
Detonations of small spherical explosive charges disrupt synaptic integrity and NMDA receptor-cofilin signaling in the absence of neurodegeneration in hippocampal slices.
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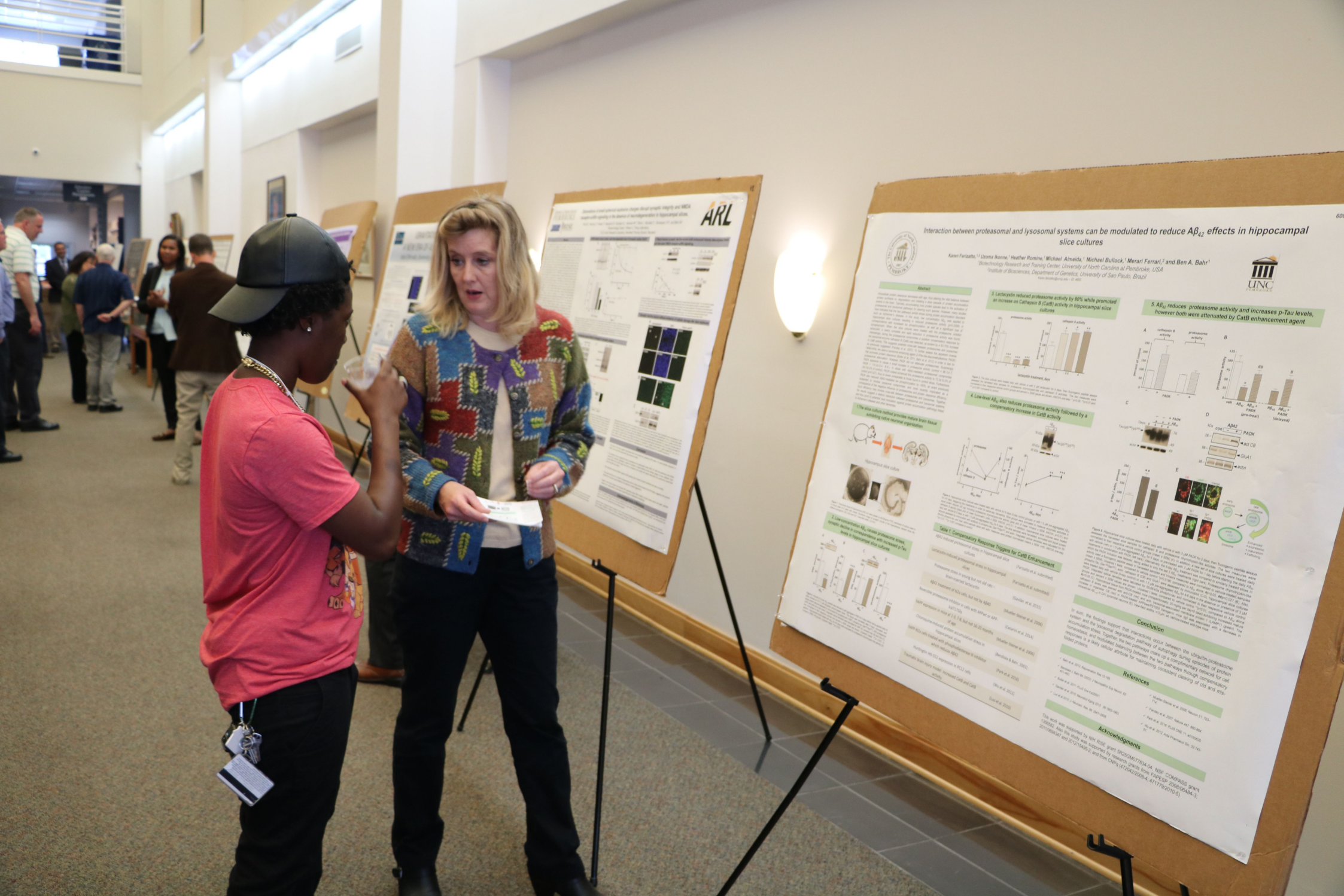
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